Crystallographic Studies of the Biologically and Medically Important Receptor-ligand and Enzyme-ligand Complexes at Advanced Light Source (ALS)

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INTRODUCTION

Our laboratory is focussed on understanding the structural basis of molecular recognition in the immune system, how cellular receptors interact with their ligands, and inhibition of medically important enzymes. To correlate their structure, function and interactions, we are investigating these molecules by x-ray crystallography. A synchrotron x-ray source at ALS offers an excellent opportunity to use small and weakly diffracting crystals and to obtain data with large unit cells. We have also conducted MAD (multiple anomalous dispersion) phasing experiments on Se-Met proteins or other heavy atom containing crystal samples, that are difficult to study with our in-house rotation anode x-ray source, and extended the resolution of the diffraction data.

EXPERIMENTS CONDUCTED AT ALS

Diffraction data on a chimeric Fab fragment of the decarboxylation catalytic antibody 21D8 in complex with the substrate analog, 5-nitro-3-caboxyindole, have been collected to 2.2Å resolution, with an R_{sym} of 7.6%. The crystal space group is $P2_12_12_1$ with cell dimensions of a=39.2Å, b=44.1Å, c=225.3Å. The structure was determined by molecular replacement with the program AMoRe using the previously determined model of the 21D8-hapten complex structure. Refinement with the program package CNS and manual rebuilding with the program O is in progress. The current R-value and R-free for all data are 24.4% and 29.7%, respectively. A manuscript is under preparation.

A highly active and all purpose aldolase antibody 33F12 Fab was crystallized in space group $P2_12_12_1$ with unit cell dimensions a=65.5Å, b=81.8Å, c=167.3Å, with two molecules in the asymmetric unit. The catalytic antibody crystals were soaked with a sulfonate inhibitor and a data set was collected to 2.0Å resolution with R_{sym}=10.7%. Molecular replacement and structure determination and refinement are currently underway.

Kaposi's sarcoma (KS) is an angiogenic tumor which is associated with herpesvirus (KSHV or HHV-8). This virus encodes chemokine-like proteins (macrophage inflammatory proteins) vMIP-I and vMIP-II. Both vMIP-I and vMIP-II partially inhibit HIV infection of peripheral blood mononuclear cells. These two viral chemokines may play the roles in the complex pathogenesis of KS. The vMIP-I native protein data were collected at 1.8Å, with the completeness of 99.2% and the R_{sym} of 8.8%. The space group of the crystal is P1 with the unit cell dimensions of a=34.2Å, b=40.6Å and c=54.5Å, alpha=83.7°, beta=89.2° and gama=79.5°. The MAD experiment of the Se-Met vMIP-I crystal of the same space group will be conducted in the future.

Diffraction data were collected for crystals containing a complex between Tissue Factor (TF) and deleted Gla contruct of Factor VIIa inhibited with EGR-chloromethylketone. The data

indicate either a 500Å long c-axis or some crystall twinning. Since these crystals are difficult to reproduce, we worked at extracting a smaller subcell from the diffraction pattern. A smaller cell in $P2_1$ space group with dimensions of a=77.7Å b=68.3Å c=70.4Å and beta=110° was found and used to integrate/scale the data, yielding a data set to 3Å with 13% R_{sym}. MR solutions were found using this reduced data set. The model currently has been refined to a R-value of 21.1% and a R-free of 34.7% with a reasonable Ramachandran plot geometry.

A native aldolase DERA that corresponds to our catalytic antibody 33F12, crystallizes in space group $P2_12_12_1$ with cell dimensions of a=50.0Å, b=53.1Å, C=177.1Å, with two molecules in the asymmetric unit. This enzyme consists of 259 residues per monomer; the 6 Met residues were substituted with Se=Met. MAD data for this enzyme were collected at 110°K during two 8-hour shifts. Complete data were collected to 1.8Å at four wavelengths at 0.97946Å 0.97969Å 0.96482Å and 0.99984Å. Ten of the 12 possible Se atom positions were determined and the structure determination is in progress.

A 2.8Å data set was also collected at ALS on a crystal of an intact IgG that is a broad and potent neutralizer of HIV-1. The space group is rhombohedral R32 and the cell dimensions are a=b=c=166.9Å and alpha=beta=gama=108.5°. Its equivalent trigonal space group with cell dimensions a=b=217.2Å, c=175.1Å, alpha=beta=90.0°, gama=120° was used for data reduction; the data are 90.9% complete with R_{sym} = 6.5%. A structural understanding of the epitope of this antibody may offer a template for vaccine design. Molecular replacement trials are currently underway.

Out of a series of T-cell receptors reactive against insulin peptide fragments presented by their restricting MHC Class II complex, IA^d, and F_v of one of these has been crystallized with a long 500Å axis; only 3.8Å data could be collected in house. A data set was collected on this large cell to around 2.3Å at ALS beamline 5.0.2 during January this year, but the resulting R_{sym} is about 20% due to the large mosaicity of the crystal. We have since then improved the quality of crystals and the cryo-conditions. The CCD detectors coupled with the possible two-theta offset at beam line 5.0.2 and the brightest beam will enable us to collect better data at higher resolution in the future.

AICAR Tfase is a folate-dependent enzyme which catalyzes both the penultimate and the last steps in the *de novo* pathway for purine biosynthesis and is thus an attractive chemotherapeutic and anti inflammatory target. The AICAR crystallizes in several different space groups with varying cell dimensions. The Se-Met AICAR crystallizes in space group $P2_1$ with cell dimensions of a=65.0Å, b=105.7Å, c=103.8Å and beta=108.4°. MAD data were collected at four wavelengths at 0.97946Å 0.97969Å 0.96482Å and 0.99984Å to 2.7Å resolution. Eighteen out of the 24 possible Se atoms in the asymmetric unit of the cell have presently been located, and MAD phasing and structure determination are currently under way.

DISCUSSION

The ALS X-ray beamline 5.0.2 and its macromolecule crystallography facility (MCF) has been a tremendous help in improving the quality and the resolution of diffraction data of our protein crystal samples. In particular, the MAD experiments that we conducted at ALS will result in a faster determination of the de novo structures. We are anticipating continued and increase use of the ALS in the future.

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